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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF ALLIUM SATIVUM ON SELECTED ORGANISMS

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Abstract

Studies of alternatives to antibiotics are of essential significance in medicine, given the prominence of antibiotics resistance of most pathogens to readily available antimicrobial agents. The research was based on the evaluation of antimicrobial activity of 70 % ethanol extract of *allium sativum* (garlic) against some selected

Introduction

Microbial pathogenicity and other infectious diseases have been controlled by use of commercially available antimicrobial drugs, since many decades. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial

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pathogens (*Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*). Using Agar Diffusion method antimicrobial susceptibility testing was conducted on the extract against the test organisms and depicted that the organisms were sensitive to the ethanolic extract of *allium sativum*, with *Staphylococcus aureus* as the most susceptible. The result showed variation of inhibition zone diameters, ranging from 7 - 15 mm, against *Salmonella typhi*, *Escherichia coli, and Staphylococcus aureus* which were dose dependent. The minimum inhibitory concentration of the garlic extract against these bacterial species was determined using Agar broth method and the result showed that the tested organisms were found to be inhibited by ethanolic extract of *Allium sativum* even at the lowest concentrations of 25%. The result of this evaluation shows that some bioactive compounds present are capable of inhibiting the growth of some pathogenic organisms, hence, can potentially contribute to resolving the issue of antimicrobial resistance.

KEYWORDS: *Allium sativum*, Ethanolic Extract, Antimicrobial Resistance, Pathogens.

Pathogens. The increasing drug resistance is the main hindrance in successful treatment of infectious diseases and the control of microbial pathogenicity (Fu, *et al*., 2007). Development of drug resistance in pathogens and increasing interest of consumers for safe food forces researchers to explore new antimicrobial agents (Erdogrul, 2002). This has given rise to a shift from the prescription of antibiotics to the use of medicinal plants and spices. It is estimated that there are about 250,000 to 500,000 species of plant on earth and relatively small percentage of them are used as food by both human and other animal species (Borris, 1996). These plants fall under the natural products which are a major source of new natural drugs and their use as an

alternative medicine for treatment of various diseases has been increased in the last few decades (Ansari *et al*., 2006). In comparison to the formulated drugs, the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available for low socioeconomic population (Adeshina *et al.,* 2011). The antimicrobial activity of spices and herbs is due to specific phytochemicals or essential oils (Avato *et al*., 2000). The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the food, pH value and temperature of the environment (Sagdic, 2003). Several reports had been published that describe the antibacterial and antifungal properties of different herbs and spices. However, there is still little information about the exact mechanism of their antimicrobial action (Gur *et al.,* 2006; Yusha'u *et al*., 2008; Belguith *et al*., 2010; Yin *et al.,* 2002; Oskay *et al*, 2009). The use of medicinal plant and spices to treat diseases of varying etiology is part of the African tradition, but in spite of thousands of years of use, not many of these bioactive plants' compounds have been exploited for clinical uses as antibiotics, though some alkaloid compounds like quinine and emetine have been developed as chemotherapeutic agents. Among those antibacterial foods that are becoming more common in western diet are green tea and ginger (Langner *et al*., 2008, Hoffman, 2007). The development of new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms. Garlic has been in use, since ancient times in India and China, for a valuable effect on the heart and circulation, cardiovascular diseases, etc (KrisEtherton, 2002; Yeh and Liu, 2001; Gardner *et al*., 2017), and regular use of garlic may help to prevent cancer, treat malaria, and raise immunity. Garlic has also been used to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella typi, Shigella spp and Staphylococcus aureus* (Teferi and Hahn, 2002). Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different micro-organisms. Therefore, this study compares the antimicrobial activity level of ginger and garlic on selected clinical pathogens.

Garlic is spice of origin but have some common chemical properties that qualify them as antimicrobial plants. However, modern bio-chemical research has discovered in it, the extra ordinary medical properties, which enable them to attack microorganisms other than human tissue. Ginger is used to cure cold, and helps to clear nasal passages.

"*Allium Sativum*" was shown to elicit macrophage (large scavenger cells) and lymphocytes (white blood cells) leading to cytotoxic destruction of tumor cells. It is further believed that garlic to be a valuable supplement to onion diet in terms of overall good health. There are many odorless garlic products available, but only one has repeatedly been proved to have medicinal and scientific merit to it.

Sebiomo, (2011) used full strength garlic juice (that 100% concentration) for every stubborn infections of fungal growth, which had become drug resistant. This is a strong antiseptic and keeps indefinitely. Garlic is a proven broad spectrum anti biotic that combats bacterial intestinal parasite and viruses. In high concentration, it cures encephalitis and discourages blood clothing. Two or three cloves a day cut the odds of subsequent heart attacks. Garlic acts as a decongested expectorant, anti-spasmodic, anti-inflammatory.

Evans (1981), reported that "garlic is proven broad spectrum anti biotic that combats bacterial intestinal parasite and viruses". In high it has cured encephalitis and discourages blood clotting.

Two or three cloves a day cut the odds of subsequent heart attacks in half, in heart patients.

Garlic reduces the inflammation of the pharynx and is very useful for tonsillitis (sore throat).

Allicin has been found to be the active ingredient in garlic and it works as an antimicrobial agent by inhibiting DNA and protein synthesis

moderately and inhibiting RNA synthesis completely as a primary target (Shobana *et al*., 2009). Garlic is also rich in anionic components such as nitrates, chlorides and sulfates and other water-soluble components found in plants and these components may have antimicrobial properties (Shobana *et al.,* 2009). Previous authors have described the antibacterial activity of garlic extract against microorganisms. Bulbs belonging to the Allium genus had the most antibacterial activity against Streptococcus mutans (Ohara *et al*., 2008) and against *Streptococcus agalactiae* (Alsaid *et al*., 2010). In addition, garlic was shown to have antimicrobial activity against *Streptococcus olaris, Streptococcus mitis*, *Staphylococcus aureus*
(Silva and Fernandes, 2010; Daka, 2011); *Escherichia coli, Salmonella typhi, Shigella flexineri,*
Proteus mirabilis (Shobana *et al*., 2009); and Vibrio parahaemolyticus, *Escherichia coli* and *Staphylococcus aureus* (Vuddhakul *et al,* 2007). Few studies have shown some bacteria to be resistant towards garlic extract and these include *Escherichia coli and Staphylococcus aureus* (Esimone *et al,* 2010)

Herbs and spices parts of plants from indigenous or exotic origin are essential part of human diet as they improve taste, color and aroma of foods (de Souza et al., 2005; Venugopal et al., 2009). In addition, they act as preservatives in many foods; they also have antioxidant (Karuppiah and Rajaram, 2012) and antimicrobial properties (Singh et al., 2008). Garlic belongs to a family of Alliaceae and its scientific name is *Allium sativum*. Other members of the family include onion, leek, shallot and leek. Garlic is widely used in culinary and medicine (Karuppiah and Rajaram, 2012). It has a pungent hot flavor but mellows and improves with cooking. It has been utilized to fight infections such as cold, cough,

asthma, diarrhea, flu, headache, sore throat, abdominal discomfort and respiratory tract infections (Abubakar, 2009; Shobana *et al.,* 2009). Food borne pathogens are widely distributed in the environment and may be a significant cause of mortality and morbidity in the population. *Escherichia coli* is a significant foodborne hazard in many countries around the world. Infection often causes hemorrhagic diarrhea, and occasionally to kidney failure and death. *Salmonella typhi* is another bacterium that is the cause of foodborne illness mainly from foods of animal origin throughout the world. *Staphylococcus aureus* cause foodborne illness due to their ability to form heat stable toxins (Sanhueza, *et al*, 2011). The present study was aimed at determining the in vitro antibacterial activity of the widely used spices namely garlic extract on the isolates of *Escherichia coli, Staphylococcus aureus and Salmonella typhi.*

MATERIALS AND METHODS

Nutrient agar, Nutrient broth, MacConkey agar, Incubator, Micropipette, Test tubes, Autoclave,
Rotary evaporator, Cork borer, Petri dish, Water bath, Evaporating dish, Wire loop, Conical flask, Measuring cylinder, Glass Funnel, Filter paper.

Methodology:

Sample Collection

Garlic (*Allium sativum*) used in this study were collected from the local Masaka market, Nasarawa state, Nigeria.

Preparation of agar:

Both Nutrient and MacConkey agar were prepared according to the measurement instructions written on them and put in a conical flask.

Both were heated until the agars were fully dissolved in the required measurement of water. After dissolving the agars, the top of a conical flask was covered with a foil paper and sterilized using an autoclave.

Microbial Strains:

Three different clinical isolates *Salmonella typhi, Staphylococcus aureus and Escherichia coli* were obtained from the Department of Pharmaceutical Microbiology, Bingham University Karu, Nasarawa state. The strains were maintained on nutrient agar and MacConkey agar slants respectively. .

Preparation of Extracts:

Ethanolic extract was prepared.by first getting a fresh garlic cloves which were shredded into shreds and air-dried for one month. After drying, garlic slices were grinded to fine powder separately using electric blender. 20g powder of garlic was soaked in 100ml of 70% ethanol. The flasks were incubated at room temperature for 72 hours. The ethanol extract was evaporated at 40°Cwith the rotary evaporator. The extract was dried using a water bath at 50°C to get exact extract in a solid form.

Using serial dilution, concentrations of 25%, 50%, 75% and 100% were gotten from the extract.

The extract solutions were stored at 4°C.

Culture Preparation:

The bacterial strains were inoculated in 10ml nutrient broth and allowed to grow overnight at 37°C separately.

Antimicrobial Activity Testing Using Agar Well Diffusion Assay:

The bacterial cultures were swabbed on the surface of sterile Nutrient agar plates using a sterile inoculating loop. Agar wells were prepared with the help of sterilized cork borer with 6mm diameter. Using a micropipette, 100ul of different concentrations of garlic extracts (25%, 50%, 75% and 100%) were added to the wells in the plate. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones was measured in cm and the results recorded.

Determination of Minimum Inhibitory Concentration (MIC):

The MIC was defined as the lowest concentration of the test compound to inhibit the growth of microorganisms

The determination of MIC of garlic extract on the test bacterial strain was done using broth dilution method as explained by Hammer et al. with different concentrations of the garlic extract. The inoculums of microorganism were prepared using 24hours cultures and suspension was adjusted to 0.5McFarland standard turbidity

Concentrations of 25%, 50%, 75% and 100% was added to each test tube containing the nutrient broth and organism. The tubes were incubated overnight at 37°C.

The test tubes were observed for turbid broth or clear broth

Determination of Minimum Bactericidal Concentration (MBC):

The **Minimum Bactericidal Concentration** (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions.

All test tubes whose concentrations gave a clear broth were carried and poured in drops on the surface of a petri dish containing 10ml nutrient and MacConkey agar this was done for 24hrs.

All agars without bacterial growth were observed, the least concentration without bacterial growth for all organisms was recorded.

PHYTOCHEMICAL SCREENING:

Alkaloids: Dangredorff's reagent was used to carry out this process. 1ml of the stock extract was measured, a capillary tube was used to spot 3 dots of the extract both horizontally and vertically on a filter paper .1ml of drangredorff's reagent was measured using a plastic dropper, and spotted on the 6 dots on the filter paper. It was left to dry for 2 minutes, after which an orange coloration was observed around the 6 dots,

Firstly, 1ml of the stock extract was measured into a test tube, and 1ml of dangredorff reagent was measured with a dropper. The dropper was put into the test tube, and 3 drops where put into the test tube, then another 3 drops were dropped slowly, until a brownish red ring was observed

Flavonoids: An alkaline test was carried out to determine the presence of flavonoids, the reagents used were NaOH & HCl. 1ml of the stock extract was measured and put into a test tube. 1ml of NaOH was measured using a dropper, and 3 drops were put into the test tube containing the stock solution. 1ml of HCl was measured using a 10ml measuring cylinder, afterwards it was turned into the test tube containing a mixture stock solution and NaOH, until a bright yellow color was observed.

Sterols &Triterpenes: Liebermann-Burchard's test was carried out to determine the presence sterols & triterpenes, and the reagents used

were acetic anhydride and concentrated H_2SO_4. Firstly, I measured 1ml of the stock extract into the test tube, 1ml of acetic anhydride was measured using a dropper, and 3 drops were put into the test tube containing the stock solution. 1ml of Conc. $H_2SO_4$was measured using a 10ml measuring cylinder, afterwards it was turned into the test tube containing a mixture stock solution and acetic anhydride, by pouring it on the side of test tube. a reddish-brown ring was observed at the interphase, with a clear bottom.

Tannins: 40% Dilute $FeCl_3$ was used as the reagent to determine the presence of tannins. Firstly,1ml of the stock extract was measured and poured into the test tube. 1ml of 40% dilute $FeCl_3$ was measured using a dropper, and 3 drops were put into the test tube containing the stock solution. No Green color observed.

Anthraquinone Glycosides: Concentrated ammonia was used as the reagent to determine the presence of anthraquinone glycosides. 1ml of the stock extract was measured and poured into the test tube. 1ml of concentrated ammonia was measured using a 10ml measuring cylinder, afterwards it was turned into the test tube, by pouring it on the side of test tube. No color change was observed.

Saponin: 1ml of the stock extract was measured and poured into the test tube, 5ml of distilled water was measured into a 25ml measuring cylinder, and turned into the test tube containing the stock extract. The top of the test tube was tightly covered with foil paper, and was shook vigorously for 20 seconds. No leather formed.

Oxalate: Resorcinol crystals, distilled water, and concentrated H_2SO_4, were used for the preliminary test for the determination of oxalates.1ml of the stock extract was measured and poured into the test tube, then I added 4 resorcinol flakes into the test tube containing the stock extract,

1ml of distilled water was also added into the test tube. The test tube was then heated using a water bath. A blue color was observed

Carbohydrates: Molisch's reagent, and concentrated H_2SO_4, were used for the preliminary test for the determination of carbohydrates.1ml of the stock extract was measured and poured into the test tube, then 3 drops of Molisch's reagent were put into the test tube containing the stock solution. 1ml of Conc. H_2SO_4 was measured using a 10ml measuring cylinder, afterwards it was turned into the test tube containing a mixture stock solution and Molisch's reagent, by pouring it on the side of test tube. No red to violet coloration was observed.

Protein: The test carried out for the preliminary test for the determination of proteins. Xanthoproteic test involves the use of concentrated HNO_3, and dilute NaOH.1ml of the stock extract was measured and poured into the test tube, 1ml of Conc. H_2SO_4 was measured using a 10ml measuring cylinder, afterwards it was turned into the test tube containing a mixture stock solution, white precipitates formed. The test tube was then heated using a water bath for 2 minutes, it was noticed that the white precipitates were insoluble after heating. The mixture was allowed to cool, then 3 drops of NaOH were added to the mixture. No change was observed. .

RESULT

Table 1: Antibacterial activity of against various selected pathogenic bacteria

Zone of inhibition measured in mm

Concentration (%)	*Escherichia coli* (mm)	*Salmonella typhi* (mm)	*Staphylococcus aureus* (mm)
25	0.0	0.0	0.0

50	7	9	10
75	11	13	14
100	14	15	17

Table 2: Minimum inhibitory concentration (MIC):

Concentration (%)	*Escherichia coli* (mm)	*Salmonella typhi (mm)*	*Staphylococcus aureus (mm)*
25	-	-	-
50	-	-	-
75	-	-	+
100	+	+	+

+=present

-=absent

Minimum bactericidal concentration (MBC): NIL

Table 3: Phyto-chemical results

PARAMETERS	RESULTS
ALKALOIDS	+
FLAVONOIDS	+
STEROLS AND TRITERPENES	-
TANINS	–
ANTHRAQUINONE GLYCOSIDES	–
SAPONIN	–
OXALATE	+
CARBOHYDRATES	–
PROTEINS	–

+=present

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_=absent

DISCUSSION:

After the tests were carried out on three different organisms*: Escherichia coli, Salmonella typhi and Staphylococcus aureus* at four different concentrations of 25%, 50%, 75% and 100% of the extract. Garlic ethanol extract was shown to be effective against all the bacteria organisms under test, the effect was seen to be greater on the increase in concentrations.

As the concentration increases it was observed that there was also increase in the zone of inhibition showing that with significant increase in concentration of extract there will yield greater results.

The minimum inhibitory concentration was seen to be 50% for the *Staphylococcus aureus* and 75% for the *Salmonella typhi* and *Escherichia coli*.

The minimum bactericidal concentration (MBC) wasn't gotten meaning the garlic ethanolic extract wasn't bactericidal.

At the end of the experiment carried out it was observed that the garlic extract had a greater effect on *Staphylococcus aureus (staph aureus)* compared to *Escherichia coli (E. coli)* and *Salmonella*.

CONCLUSION

The results obtained in this study showed an explanation for the relatively higher therapeutic efficacy of spices. Garlic has antibacterial activity. Garlic is seen to have a good activity on both Gram-positive and Gram-negative bacteria.

Garlic can be used in the prophylaxis treatment for disease caused by some of these organisms, it can also be used as an ingredient in herbal formulations.

There are several advantages for the use of spices as dietary supplement or alternative medicine manifested by reduction the chance for developing antibiotic resistant bacteria that resulted from the frequent use of antibiotic, besides decreasing the cost of treatment and also minimizes the development of adverse drug reactions.

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